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Multicenter Clinical Evaluation of the Novel Alere i Strep A Isothermal Nucleic Acid Amplification Test

Daniel M. Cohen,[a] Michael E. Russo,[a] Preeti Jaggi,[b] Jennifer Kline,[a] William Gluckman,[c] Amisha Parekh[d]

Division of Emergency Medicine, Nationwide Children's Hospital, Columbus, Ohio, USA[a]; Section of Infectious Diseases, Nationwide Children's Hospital, Columbus, Ohio, USA[b]; FastER Urgent Care, Morris Plains, New Jersey USA[c]; New York Methodist Hospital, Brooklyn, New York, USA[d]

Rapid detection of group A beta-hemolytic streptococcus (GAS) is used routinely to help diagnose and treat pharyngitis. However, available rapid antigen detection tests for GAS have relatively low sensitivity, and backup testing is recommended in children. Newer assays are more sensitive yet require excessive time for practical point-of-care use as well as laboratory personnel. The Alere i strep A test is an isothermal nucleic acid amplification test designed to offer highly sensitive results at the point of care within 8 min when performed by nonlaboratory personnel. The performance of the Alere i strep A test was evaluated in a multicenter prospective trial in a Clinical Laboratory Improvement Amendments (CLIA)-waived setting in comparison to bacterial culture in 481 children and adults. Compared to culture, the Aleri i strep A test had 96.0% sensitivity and 94.6% specificity. Discrepant results were adjudicated by PCR and found the Alere i strep A test to have 98.7% sensitivity and 98.5% specificity. Overall, the Alere i strep A test could provide a one-step, rapid, point-of-care testing method for GAS pharyngitis and obviate backup testing on negative results.

In developed countries, 15% of school-age children and 4% to 10% of adults will have an episode of group A streptococcus (GAS) pharyngitis each year (1). The accurate diagnosis and treatment of GAS pharyngitis is necessary to reduce symptoms, prevent suppurative and nonsuppurative sequelae, reduce transmission, and avoid unnecessary antibiotic use (1). Yet, clinical features and prediction rules alone are not adequate to make a diagnosis (2). Given that current point-of-care rapid antigen detection tests (RADT) for GAS have a limited sensitivity of approximately 85% (3), current guidelines recommend confirming negative results in children with a backup method, most commonly by culture (1). However, this two-step process adds additional time and costs, and it risks that some patients will be lost to follow-up (4). The Alere i strep A test is a new isothermal nucleic acid amplification test using a nicking enzyme amplification reaction (NEAR) that can provide results in under 8 min in a point-of-care (POC) setting. We performed a prospective, multicenter trial to evaluate the performance of the Alere i strep A test in comparison to bacterial culture, as the reference standard, with PCR adjudication for discrepant results.

MATERIALS AND METHODS

We prospectively enrolled subjects at 10 clinical sites within the United States (in Florida, Georgia, Nebraska, New Jersey, New York, and Ohio) from 21 January to 14 March 2014. The sites included emergency departments (general and pediatric), an urgent care center, and private practices (clinical and research focused). Subjects were eligible if they presented with complaints of a sore throat and signs of suspected pharyngitis: pharyngeal erythema, tender cervical lymphadenopathy, swollen tonsils, palatal petechiae, or scarlatiniform rash. Subjects were excluded if they had systemic antibiotic use in the past 2 weeks, had previously participated in the study, were enrolled in a clinical drug trial, or were part of a vulnerable population deemed inappropriate. We obtained informed consent for subjects ≥18 years old or parental informed consent for subjects <18 years old, with patient assent for subjects 7 or 9 to 17 years old, as per the institutional review board approval that was obtained by each site. Each subject had a set (2) of swabs of their posterior oropharynx obtained for the study. The Alere i strep A kit contains foam tip swabs. Alternately, polyester, rayon, and nylon flocked-tipped swabs may be used. Calcium alginate swabs may not be used.

Alere i strep A. For the study, one swab was processed using the Alere i strep A platform by untrained nonlaboratory personnel according to the manufacturer's instructions in a Clinical Laboratory Improvement Amendments (CLIA)-waived setting. Test operators included physicians, physician assistants, and research assistants. The platform consists of a reusable Alere i instrument base and a single-use sample receiver, test base, and transfer cartridge (see Fig. 1). The test base contains two sealed reaction tubes (one as a positive control), each containing a lyophilized pellet containing the necessary reagents for the nicking enzyme amplification reaction. The template is targeted against the cell envelope proteinase A (*cepA*) gene.

To run the Alere i strep A test, the patient information is entered into the instrument base. The sample receiver and test base are then each inserted into the instrument base and heated for 2 min. The specimen is then eluted in the sample receiver for 10 s and transferred via the transfer cartridge into the test base. The instrument base then performs amplification and detection by fluorescent molecular beacon probes. A specimen is called positive as soon as it crosses an internal algorithm-defined threshold for detection; this may take only several minutes. Negative results are returned after 6 min of attempted amplification. Overall, the test requires less a minute of hands-on time (entering the patient information, inserting the sample receiver and test base, eluting the sample, and transferring the sample into the test base.) It must be attended twice: during the initial setup and then 2 min later to elute and transfer the sample into the test

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Address correspondence to Daniel M. Cohen, daniel.cohen@nationwidechildrens.org.

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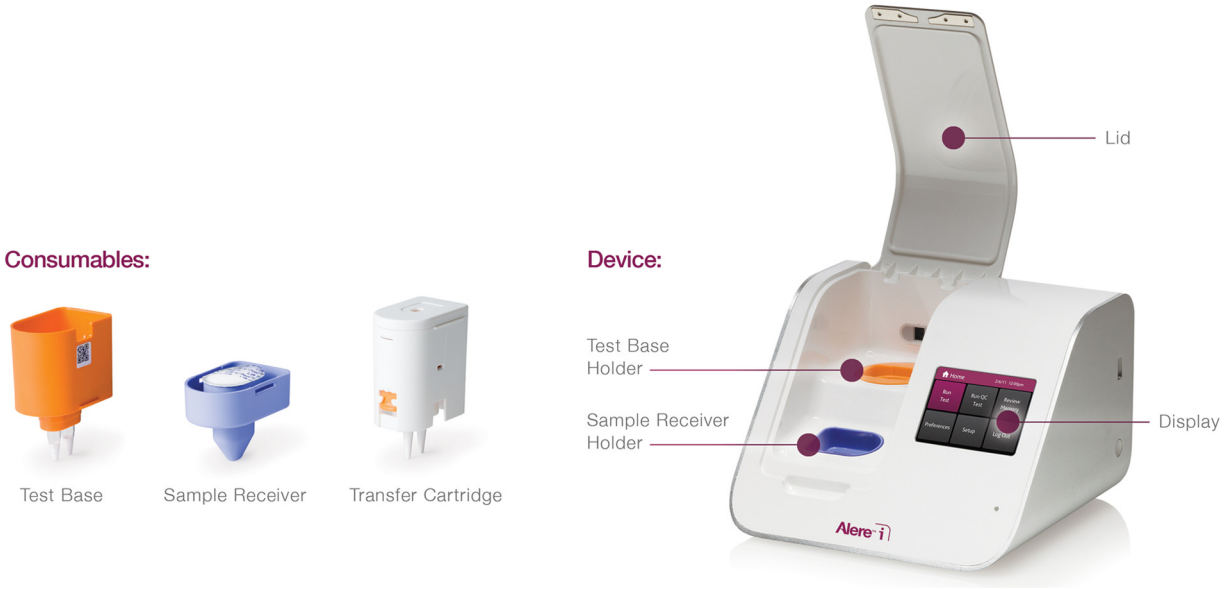

FIG 1 Overview of the Alere i strep assay.

base. The turnaround time is 8 min for negative results and less than 8 min if the results are positive. If none of the amplification products are detected, including the internal control, the test is reported as invalid. For this study, results were considered invalid if two attempts on the same specimen yielded invalid results. Additional control testing with positive- and negative-control swabs was performed daily prior to sample processing.

Bacterial culture. The second study swab was cultured at a central clinical laboratory within 48 h of collection. The specimens were inoculated on Becton, Dickinson TSA II sheep blood agar and incubated for 24 h at 37°C without CO_2 enrichment. Plates with no visible hemolysis were reincubated at room temperature for an additional 24 h. Anaerobic culture was not performed. Bacterial culture results were considered positive if GAS was isolated. Cultures were considered negative if there was no growth or if any bacterium other than GAS was isolated (including non-group A beta-hemolytic or nongroupable streptococci). Streptococci were typed using latex agglutination with the PathoDx strep grouping kit.

Real-time PCR analysis. The swab used for plate inoculation was frozen for later PCR testing if there were discordant findings between the Alere i strep A test and bacterial culture (positive-negative or negative-positive results). Only these discordant samples were tested by PCR. The Primerdesign genesig kit targeted against the *sc1A* gene (5) was used according the manufacturer's instructions.

McIsaac scores. The McIsaac score (6) is a clinical prediction rule suggested by the American Academy of Pediatrics (AAP) (7) to help identify which patients with sore throats should undergo diagnostic testing for GAS. It assigns one point each for a history of or a documented fever (38°C), lack of cough, tender anterior cervical adenopathy, tonsillar swelling or exudates, and age <15 years; a point is subtracted for age >44 years. Scores range from −1 to 5; patients with a score of 2 or higher are recommended to be tested for GAS.

Statistical analysis. Test data were summarized in 2 × 2 tables. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and their associated confidence intervals (CIs) were determined based on calculated proportions by the Wald method. The Fisher's exact test with a P value of <0.05 was used as the cutoff to test the associations between test characteristics and age group or setting. SAS statistical software (version 9.3) was used.

RESULTS

We enrolled a total of 505 subjects. Ten subjects were excluded following enrollment: 5 subjects, due to delayed sample delivery (>48 h) to the central lab due to inclement weather; 4 subjects, because of sample mishandling; and 1 subject, for failure to meet inclusion/exclusion criteria. Fourteen (2.8%) of the total samples had invalid Alere i strep A results and were excluded. A total of 481 subjects were retained for the final analysis. The median age was 11 years (interquartile range [IQR], 7 to 19 years). Twelve subjects were <3 years of age; 62% of the cohort was female. Forty-six percent of subjects were seen in an emergency department or urgent care setting (ED/UC). The prevalence of GAS by culture was 30.3% overall, 36% in children <18 years, and 14% in adults. The prevalence of GAS by culture result was 8.5%, 30.2%, 43.4%, and 65.3% for subjects with McIsaac scores of ≤1, 2, 3, and ≥4, respectively. GAS prevalence by the Alere i strep A test was 9.9%, 35.2%, 44.3%, and 65.3% for subjects with the same respective

TABLE 1 Performance of Alere i strep A test versus culture[a]

Age group	No. of swabs with: TP	FP	TN	FN	Total no. of swabs	Sensitivity (% [95% CI]	Specificity (% [95% CI])	PPV (% [95% CI])	NPV (% [95% CI])
<18 yr	123	15	212	5	355	96.1 (92.7–99.5)	93.4 (90.2–96.6)	89.1 (83.9–94.3)	97.7 (95.7–99.7)
≥18 yr	18	3	104	1	126	94.7 (84.7–104.8)	97.2 (94.1–100.3)	85.7 (70.8–100.7)	99.1 (97.2–100.9)
Combined	141	18	316	6	481	95.9 (92.7–99.1)	94.6 (92.2–97.0)	88.7 (83.8–93.6)	98.1 (96.7–99.6)

[a] TP, true positive; FP, false positive; TN, true negative; FN, false negative; CI, confidence interval.

TABLE 2 Performance of Alere i strep A test versus culture following PCR adjudication of discrepant results[a]

Age group	No. of swabs with: TP	FP	TN	FN	Total no. of swabs	Sensitivity (% [95% CI])	Specificity (% [95% CI])	PPV (% [95% CI])	NPV (% [95% CI])
<18 yr	134	4	215	2	355	98.5 (96.5–100.6)	98.2 (96.4–100.0)	97.1 (94.3–99.9)	99.1 (97.8–100.4)
≥18 yr	20	1	105	0	126	100 (100.0–100.0)	99.1 (97.2–100.9)	95.2 (86.1–104.4)	100 (100.0–100.0)
Combined	154	5	320	2	481	98.7 (97.0–100.5)	98.5 (97.1–99.8)	96.9 (94.1–99.6)	99.4 (98.5–100.2)

[a] TP, true positive; FP, false positive; TN, true negative; FN, false negative; CI, confidence interval.

McIsaac scores. Compared to culture, the Alere i strep A test had 95.9% sensitivity and 94.6% specificity (Table 1). Following discrepant analysis by PCR, the novel test had 98.7% sensitivity and 98.5% specificity (Table 2). There was no statistically significant association between test characteristics and age (<18 years versus ≥18 years, $P = 0.57$ and 0.2 for sensitivity and specificity, respectively) or enrollment setting (ED/UC versus office based, $P = 0.70$ and 0.92 for sensitivity and specificity, respectively).

In the 13 subjects whose specimens were positive by PCR and Alere i strep A but negative by GAS culture, there was no growth of any beta-hemolytic streptococcal species in 12 subjects, and there was growth of group G streptococcus in 1 subject. Eleven of these 13 subjects had McIsaac scores of 2 or 3; the remaining 2 subjects had McIsaac scores of 1. Lancefield typing of non-group A beta-hemolytic streptococci identified groups B ($n = 3$), C ($n = 3$), F ($n = 5$), and G ($n = 3$) and nongroupable ($n = 12$) beta-hemolytic streptococci. The Alere i strep A test and PCR were concurrently positive for 1 subject with group G streptococcus and 1 with nongroupable streptococcus but for none of the others. No specimens grew *Arcanobacterium haemolyticum*.

DISCUSSION

Current rapid antigen detection tests are inadequate in most settings to completely rule out GAS pharyngitis in children without a backup method (1). Although bacterial culture and PCR are considered the two most-sensitive tests for GAS pharyngitis, they are time consuming and require additional expertise. Although there are other newer assays with test characteristics that could potentially be used for one-step GAS testing in children, these also require trained laboratory personnel and equipment and are not rapid enough to support efficiency in emergency department, urgent care center, or outpatient practices (8, 9).

In the present study, we found that the Alere i strep A test, with a sensitivity of 95.9% and a specificity of 94.6% compared to bacterial culture and the ability to be performed in ~8 min, favorably addresses both of the above issues. Additionally, in this study, we used PCR to adjudicate discrepancies between bacterial culture and the Alere i strep A platform, with a resultant sensitivity and specificity of 98.7% and 98.5%, respectively. Bacterial culture is the most commonly used reference standard for detecting the presence of GAS in throat specimens. However, patients with small or no GAS growth on specimen culture, possibly due to shipping, low inoculum, or bacterial inhibition, (10), may still have serologic responses, (11) which suggests limitations of culture as the reference standard. PCR has thus been suggested as an alternate reference standard (10, 12). By example, 13 of our subjects had no GAS growth yet had positive results by both the Aleri i strep A test and PCR. Given that the reference PCR test used in this study and the Alere i strep A test target different genes, GAS genetic material was likely present. The presence of nucleic acid may represent active GAS pharyngitis, resolving infection with residual nucleic acid but no viable strep present, or carriage. As 11 of these subjects had McIsaac scores of 2 or 3, some portion of these subjects could be reasonably expected to have had true GAS infections and would represent additional cases found by the Alere i strep A test. On the other hand, conceivably, nucleic acid amplifications tests may detect a clinically insignificant presence of GAS. This may have been the case in the 2 subjects with McIsaac scores of 1. The specimens of 4 subjects were positive by culture yet negative by the Alere i strep A test and PCR. This could be due to the concurrent failure of each of the nucleic acid amplification tests or an error in Lancefield typing. We did not perform PCR on all specimens; this limitation precludes a direct comparison of the Alere i strep A test to PCR, a potential future study.

Fourteen of the 505 subjects had invalid results on the Alere i strep A test. Specific reasons for the invalid results were not identified; however, given that 10 of the 14 invalid results occurred at a single site, it is possible that operator error or failure of a single test base could have contributed. This invalid rate is similar to that found in a study of the Alere i influenza test which uses the same instrument base (13). In practice, if a patient had an invalid result despite repeat testing, a clinician could instead use a throat culture or a different nucleic acid-based method.

The Alere i strep A test is limited, like all diagnostic tests for GAS pharyngitis, in the inability to distinguish between colonization and infection. Similar to all tests, excluding throat culture, the Alere i strep A test also cannot be used to evaluate antibiotic resistant patterns, evaluate type strains, or detect alternative bacterial causes of pharyngitis. The test demonstrated an excellent negative predictive value despite a relatively high prevalence of GAS in this study. Given the dramatic decline in the circulation of rheumatogenic strains of GAS since the 1960s, (14) a single-step testing method using a highly sensitive assay such as this novel platform might obviate backup testing in children. Practically, the current platform runs one test at a time; thus, it is best suited to POC use as opposed to high-volume batch processing.

Overall, the test performs equally well in children and adults and is easily performed by nonlaboratory personnel in a variety of clinical settings.

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We have submitted the ICMJE form for disclosure of potential conflicts of interest and report no potential conflicts.

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